Eyler Yvonn
09/016,74

7) Pattison et al.
"The RANTES chemokine. A new target for immunomodulatory therapy?"
CLINICAL IMMUNOTHERAPEUTICS
Vol. 4 (1)
p. 1-8
1995

8) Mule et al.
"RANTES secretion by gene-modified tumor cells results in loss of tumorigenicity in vivo..."
HUMAN GENE THERAPY
vOL. 7 (13)
P. 1545-1553
1996

9) Cardoso et al.
"Lymphoblastic leukemia cells express CXCR-4 and migrate through endothelium in response to SDF-1..."
BLOOD
Vol. 92 (10 suppl. 1 part 1-2)
p. 618A
Nov. 15, 1998

10) Schall, T.
"Fractalkine--a strange attractor in the chemokine landscape"
IMMUNOLOGY TODAY
Vol. 18 (4)
p. 147-?
1997

11) Dilloo et al.
"combined chemokine and cytokine gene transfer enhances antitumor immunity"
NATURE MEDICINE
Vol. 2 (10)
p. 1090-1095
1996

12) Tannenbaum et al.
"Anti-tumor functions of IP-10 and Mig"
JOURNAL OF LEUKOCYTE BIOLOGY
Suppl
p. 18-?
1997

13) Oppenheim et al.
"Prospects for cytokine and chemokine biotherapy"
CLINICAL CANCER RESEARCH
Vol. 3 (12)
p. 2682-2686
1997

14) Nakashima et al.
"Human MCAF gene transfer enhances the metastatic capacity of a mouse cachectic adenocarcinoma cell line in vivo"
PHARMACEUTICAL RESEARCH
Vol. 12 (11)
p. 1598-1604
1995

15) Kruger et al.

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COMPLEMENT, CHEMOKINES AND ACUTE LUNG INJURY.
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Acute inflammation leading to lung injury is known to be complement-dependent, although the basis for this relationship is not well understood. Using intrapulmonary deposition of IgG immune complexes in rat lungs, which induces a prompt and vigorous damaging inflammatory response, we have recently demonstrated the requirements for CXC (CINC, MIP-2) and CC (MIP-1α) chemokines. In the current studies, using the same lung model, complement depletion (by intraperitoneal injections of cobra venom factor) or C5a blockade (by anti-rat C5a) significantly reduced the levels of chemokines appearing in bronchoalveolar (BAL) fluids. Conversely, intratracheal instillation of either C5a or the membrane attack complex (C5b-9, MAC), while alone not causing chemokine expression, together with immune complexes caused significantly increased levels of chemokines in BAL fluids, increased numbers of neutrophils, and accentuation of lung injury. In *vitro* we have shown that the copresence of C5a or MAC with immune complexes (or bacterial lipopolysaccharide) causes synergistic increases in production of chemokines by rat alveolar macrophages. These data indicate linkages between complement activation products, chemokine production, and lung damaging acute inflammatory responses. In the clinical setting, it is possible that chemokine production *in vivo* can be controlled by manipulation of the complement system.

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STEM CELL FACTOR ACTIVATES BOTH THE JAK/STAT AND SRC FAMILY PATHWAYS IN HEMATOPOIETIC CELLS.
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Stem cell factor (SCF) is a growth factor important in hematopoiesis. The receptor for SCF is c-Kit, a receptor tyrosine kinase (RTK). The objective of this study was to delineate novel signal transduction components activated by SCF. Our previous studies have suggested that JAK2 plays a role in SCF-mediated proliferation. Recently we examined the transcription factor Stat1 in relation to SCF signal transduction and found that SCF induced association of Stat1 and c-Kit, tyrosine phosphorylation of Stat1 and activation of Stat1 DNA binding activity. Further, we have found that the Stat1 SH2 domain interacts with phosphorylated tyrosine residues within the catalytic domain of c-kit. We have also examined the effect of SCF on Src family members. Stimulation of cells with SCF resulted in rapid activation of Lyn kinase activity. Lyn coimmunoprecipitated with c-Kit and this association was mediated through the phosphorylated c-Kit juxtamembrane region. Treatment of cells with Lyn antisense oligonucleotides or PP1, a Src family inhibitor, dramatically inhibited SCF-induced proliferation. These data demonstrate that SCF activates both the JAK/STAT and the Src family pathways and suggests these pathways play a role in SCF-mediated responses.

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ROLE OF STEROIDS IN THE DIFFERENTIATION OF STEM CELL FACTOR DEPENDENT RAT MYELOMONOCYTIC PROGENITORS.
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Glucocorticoids (GC) and 1 α,25-dihydroxyvitamin D3 (Vit.D3) are known to reduce proliferation of normal progenitors and of leukemic cell lines and promote their differentiation into granulocytes (Gr) and macrophages (MΦ), respectively. However, it is still unclear whether this is a direct effect on the hemopoietic cell or is mediated through an enhancement of cytokine (CK) production. Now we have established highly homogeneous cultures of non-transformed myelomonocytic progenitor cells from rat bone marrow by initial stimulation with a critical concentration of wheat germ agglutinin followed by stem cell factor (SCF). These cells have a blast-like morphology, lack most lineage specific markers, express high levels of CD45 and CD90 and can be propagated with SCF for at least 50 generations. When SCF is withdrawn these cells rapidly undergo apoptosis which cannot be prevented by the addition of any other hematopoietic CK. When such CKs are added to cultures optimally stimulated by SCF only G-CSF is able to enhance proliferation. None of the CKs leads to any differentiation. However, addition of GC induces differentiation into mature Gr within 3 to 5 days, whereas addition of Vit.D3 leads to differentiation into predominantly MΦ within 5 to 7 days. Retinoic acid had no direct differentiation inducing effect but led to nuclear hypersegmentation of GC-induced Gr. These results demonstrate that GC and Vit.D3 are necessary and sufficient to induce terminal differentiation into granulocytes and macrophages, respectively.

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The DSL Domain of Mouse Delta Homologue Dll1 Modulates Hematopoietic Cell Responses to Hematopoietic Growth Factors
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Delta and *Notch* encode cell surface proteins that influence a wide variety of cell fate decisions in the development of *Drosophila*. Signals generated by interaction of Delta ligand and Notch receptor can affect the cell competence in response to further developmental cues. Vertebrate homologues of Delta and Notch were identified and shown to be essential for normal embryonic development. However functions of vertebrate homologues of Delta and Notch in regulation of hematopoietic cells in response to hematopoietic growth factors (HGFs) is poorly understood. We report here analysis of the role of a murine *Delta* homologue, *Delta-like 1 (Dll1)*, in regulation of hematopoiesis in response to HGF stimulation. The expression of *Dll1* protein (Dll1) in adult mouse bone marrow (BM) was detected as an 80 kD protein by Western blot analysis with an affinity-purified anti-Dll1 antibody. BM cells were isolated from a $Dll1^{lac\text{-}Z}$ mouse. β-galactosidase staining of the isolated cells showed that stromal/endothelial cells of BM expressed Dll1. Furthermore, we have demonstrated a role of Dll1 in regulation of hematopoiesis using a soluble recombinant form of the extracellular DSL domain of Dll1 produced in *E. coli*. DSL enhanced the expansion of primitive hematopoietic precursors (high proliferation potential colony-forming cells - HPP-CFCs) when combined with HGFs including IL-3, G-CSF or GM-CSF. DSL promoted the expansion of primitive HPP-CFC responsive to IL-3, IL-1β and M-CSF, and at the same time inhibited the differentiation of primitive precursors into more mature precursor cells responsive to IL-3 alone. Culture of post-5-fluorouracil BM, or of purified BM precursors, with DSL plus IL-3 led to generation of immature promonocytes and their prolonged proliferation for up to nine weeks with inhibition of their terminal macrophage differentiation. DSL also synergized with specific HGFs to prevent apoptosis of hematopoietic cells in BM suspension cultures. Our data suggest that DSL, most likely by activating the Notch1 receptor expressed in hematopoietic cells, modulates cellular competence to respond to conventional HGFs by selectively blocking HGF differentiation, but not proliferation signals.

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Recognition of Phosphatidylserine on Apoptotic Cells By Human Macrophages Is Associated With CD36 And With Inhibition of Proinflammatory Cytokine Secretion
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Phagocytosis of apoptotic cells prior to lysis is critical for tissue remodeling and resolution of inflammation. Human macrophages (HMDM) utilize the vitronectin (CD51/61) and thrombospondin (CD36) receptors to engulf apoptotic cells. Mouse bone-marrow-derived macrophages also use the VnR; however, they can be stimulated with glucan to recognize phosphatidylserine (PS) on apoptotic cells in a manner suggestive of a receptor. Switch to PS recognition is accompanied by loss of VnR usage, but not expression. We therefore treated HMDM with glucan to determine if a similar switch in recognition was induced. Apoptotic cell uptake by unstimulated HMDM was inhibited by RGDS, anti-VnR, and anti-CD36, but not by PS. By contrast, HMDM treated with glucan for 48 hours were inhibited by PS and its structural analogues, but not RGDS or anti-VnR. Anti-CD36 remained inhibitory. PS liposomes failed to inhibit the binding of anti-CD36 to either unstimulated or glucan-stimulated macrophages, as assessed by flow cytometry. However, down-regulation of CD36 on the surface of stimulated macrophages was associated with a loss of PS inhibition of phagocytosis. Phagocytosis of apoptotic cells by either unstimulated or glucan-stimulated macrophages suppressed LPS-induced proinflammatory cytokine production. These data suggest that CD36 is involved in recognition of PS on apoptotic cells by glucan-stimulated macrophages, although it may not bind PS per se. This recognition mechanism, like the VnR/CD36 mechanism, is associated with inhibition of inflammatory cytokine secretion.

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Anti-tumor functions of IP-10 and Mig C. S. Tannenbaum, R. Tubbs, D. Armstrong, J. Finke, R. Bukowski and T. Hamilton. Lerner Research Institute, Department of Immunology, Cleveland Clinic Foundation, Cleveland, Ohio.

The role of the non-ELR containing CXC chemokines IP-10 and Mig in anti-tumor activity was examined in mice bearing the renal adenocarcinoma RENCA. Treatment with IL-12 produces a potent anti-tumor effect which is associated with tumor infiltration by CD8+ T lymphocytes. The regression of tumor is associated with the elevated expression of the IFNγ-inducible chemokines IP-10 and Mig within the tumor tissue. In animals treated with rabbit polyclonal antibodies specific for IP-10 and for Mig, the IL-12-induced regression of RENCA tumors was partially abrogated and was associated with a substantial inhibition of T cell infiltration. Tumors engineered to express Mig grew normally in vitro but exhibited markedly reduced growth in vivo as compared to parental tumors. This anti-tumor effect was associated with intratumoral fibrosis suggesting the recruitment and/or expansion of host-derived tumor stroma. This effect was independent of T cell recruitment. Thus it appears that anti-tumor activity associated with Mig and/or IP-10 involves both T cell-dependent and -independent functions.